Analytica Chimica Acta 813 (2014) 63–69

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Quantitative solid phase microextraction – Gas chromatography mass spectrometry analysis of five megastigmatrienone isomers in aged wine[☆]

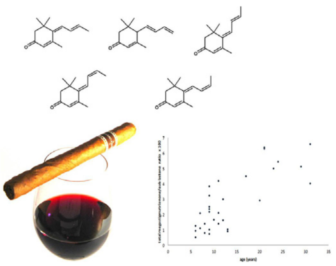
Davide Slaghenaufi[a,b,*], Marie-Claire Perello[a,b], Stéphanie Marchand-Marion[a,b], Sophie Tempere[a,b], Gilles de Revel[a,b]

[a] *Univ. Bordeaux, ISVV, EA 4577, Unité de Recherche Œnologie, 33882 Villenave d'Ornon, France*
[b] *INRA, ISVV, USC 1366 Œnologie, 33882 Villenave d'Ornon, France*

HIGHLIGHTS

- The first SPME–GC–MS method was developed for simultaneous quantification of five megastigmatrienone isomers in wine.
- The method was completely validated for white and red wines.
- The five megastigmatrienone isomers were quantified in 65 wines.
- Megastigmatrienone concentration increase with wine age.
- Aging wine in oak barrels enrich wine in megastigmatrienone.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 September 2013
Received in revised form 3 January 2014
Accepted 9 January 2014
Available online 15 January 2014

Keywords:
Megastigmatrienone
Mass spectrometry
Gas chromatography
Solid phase microextraction
Wine
Tobacco aroma

ABSTRACT

Megastigmatrienone is a key flavor compound in tobacco. It has also been detected in wine, where it may contribute to a tobacco/incense aroma, but its importance and concentration in wines had never previously been evaluated.

A method was developed and validated for quantifying the five megastigmatrienone isomers in red and white wines.

Megastigmatrienone isomers were extracted by headspace solid-phase microextraction (HS-SPME), with a 65 μm film thickness polydimethylsiloxane–divinylbenzene (PDMS–DVB) fiber and analyzed using gas chromatography–mass spectrometry (GC/MS) in selected ion monitoring mode (SIM). Several parameters affecting the length of the adsorption process (i.e., adding salt, extraction time and extraction temperature) were tested. The optimum analytical conditions were established.

The LOQ were between 0.06 $\mu g\,L^{-1}$ and 0.49 $\mu g\,L^{-1}$ for white wine and 0.11 $\mu g\,L^{-1}$ and 0.98 $\mu g\,L^{-1}$ for red wine, repeatability in both types of wine was less than 10% and recovery ranged from 96% for white wine to 94% for red wine. The five isomers of megastigmatrienone were quantified in red and white wines for the first time. Concentrations ranged from 2 $\mu g\,L^{-1}$ to 41 $\mu g\,L^{-1}$ in both red and white wines. Initial results revealed a link between wine aging and megastigmatrienone levels, indicating that megastigmatrienone may be a component in wine "bouquet".

[☆] Selected paper presented at In Vino 2013 – Reims, France from 3 to 5 July 2013.

[*] Corresponding author at: Univ. Bordeaux, ISVV, EA 4577, Unité de Recherche Œnologie, 33882 Villenave d'Ornon, France. Tel.: +33 557 575 863; fax: +33 557 575 803.
E-mail address: davideslaghenaufi@gmail.com (D. Slaghenaufi).

0003-2670/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aca.2014.01.019

1. Introduction

Megastigmatrienone, known to be a key aroma compound in Burley tobacco [1], has a sweet tobacco aroma, with spicy and peppery notes [2–4]. Megastigmatrienone has two conjugated double bonds on the butenyl side chain, resulting in 4 diasteroisomers (megastigma-4,6*Z*,8*Z*-trien-3-one, megastigma-4,6*E*,8*Z*-trien-3-one, megastigma-4,6*Z*,8*E*-trien-3-one, megastigma-4,6*E*,8*E*-trien-3-one) and one position isomer (megastigma-4,7*E*,9-trien-3-one), see Fig. 1.

α-Megastigmatrienone was previously detected in Cabernet Sauvignon wine [5,6] and, more recently, Graciano [7]. Three of the five isomers: megastigma-4,6*Z*,8*E*-trien-3-one, megastigma-4,6*E*,8*E*-trien-3-one [8], and megastigma-4,7,9-trien-3-one [9], have been identified in fresh oak wood.

Little is known about the origin of this compound in oak wood and still less about its presence in wine, where it has only been detected once. One possible source of megastigmatrienone is carotenoid breakdown [9,10] and it has been demonstrated that the five isomers may be formed by dehydration of 3-oxo-α-ionol under mildly acidic conditions [11]. In wine, megastigmatrienone may be produced hydrolysis and dehydration of the 3-oxo-α-ionol naturally present in grapes, mainly as a glycoconjugate precursor [12]. However, while the conversion of 3-oxo-α-ionol into megastigmatrienone is quite rapid at pH 1 and warm temperatures, the reaction is apparently quite slow at pH 3, the typical value in wine [12]. Macarangioside E was recently identified in oak wood for the first time [13]. This compound is not only the galloyl-glucoside precursor of 3-oxo-α-ionol, but was also demonstrated to be capable of forming all five megastigmatrienone isomers when subjected to heat treatment without acid catalysis [13]. Consequently, toasted oak barrels could enrich wines in megastigmatrienone, thus contributing to the complexification and intensification of aromas in older wines commonly known as their “bouquet”.

Megastigmatrienone was detected in wine using several extraction techniques applied principally to wine profiling, such as HS-SPME coupled with GCxGC–TOF [6] and SBSE–GC–MS [5,7]. The concentration in Cabernet Sauvignon wines was estimated at less than 5 μg L^{-1} [5], but no validated quantification method has been developed for analyzing wine or other beverages, nor to our knowledge, for other matrices, such as tobacco.

This paper reports the development and validation of a headspace solid-phase microextraction gas-chromatography mass-spectrometry quantification method (HS-SPME–GC–MS) for the five megastigmatrienone isomers.

The HS-SPME technique is now widely used for flavor analysis in food, as it is fast, economical, solvent-free, and capable of detection at low thresholds [14]. It has been widely applied for analyzing flavors in wine [15–20] and volatile compounds in oak barrels [21,22].

65 white and red wines from different countries, varieties, and ages were then analyzed using the method developed. A comparison of concentrations with wine age highlighted a link between wine aging and the appearance of megastigmatrienone, indicating that megastigmatrienone may be a component of qualitative wine aging aroma, commonly known as wine “bouquet”.

2. Materials and methods

2.1. Reagents and materials

Megastigmatrienone was kindly provided by Symrise AG (Holzminden, Germany) as a mix of isomers: 11% megastigma-4,6*Z*,8*E*-trien-3-one, 32% megastigma-4,7*E*,9-trien-3-one, 35% megastigma-4,6*Z*,8*Z*-trien-3-one, 4% megastigma-4,6*E*,8*E*-trien-3-one, and 18% megastigma-4,6*E*,8*Z*-trien-3-one. Dodecan-2-ol at 98% purity, used as an internal standard, was supplied by (Acros organic). Milli-Q quality water (18.2 MΩ cm) was produced from distilled water by a Milli-Q Plus water system (Millipore, Saint-Quentin-en-Yvelines, France).

2.2. Wine samples

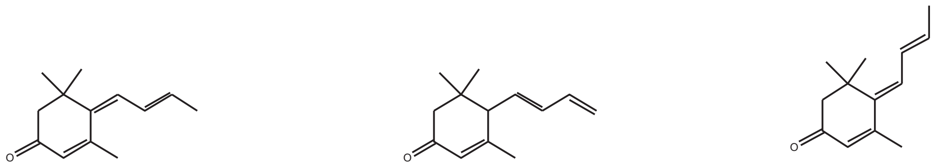
A white wine (Chardonnay) and a 2011 Bordeaux AOC red wine (Merlot) were used to optimize analytical conditions. The two wines were spiked with 100 μg L^{-1} of the megastigmatrienone isomer blend. After optimization and validation of the method, 65 wines from various regions, countries, and vintages were analyzed. All optimization, validation and quantification analyses were performed in triplicate.

2.3. Sample preparation

The pH of a 30 mL sample of wine was raised to pH 7 using 10 M, 1 M, and 0.1 M NaOH, 10 ml samples were collected and placed in a 20 mL vial containing 3.5 g sodium chloride and diluted with 10 mL MQ water, spiked with 10 μL internal standard (dodecan-2-ol at 400 mg L^{-1}), and the vial was sealed with a PTFE-lined cap (Supelco, Bellefonte, PA, USA).

2.4. HS-SPME extraction

Several fiber coatings (composition of stationary phases and film thicknesses) were tested (Supelco, Bellefonte, PA, USA):



megastigma-4,6*Z*,8*E*-trien-3-one megastigma-4,7*E*,9-trien-3-one megastigma-4,6*E*,8*E*-trien-3-one

megastigma-4,6*E*,8*Z*-trien-3-one megastigma-4,6*Z*,8*Z*-trien-3-one

Fig. 1. Structure of five megastigmatrienone isomers.